State of Hawai'i DEPARTMENT OF LAND AND NATURAL RESOURCES

Division of Forestry and Wildlife Honolulu, Hawai'i 96813 May 26, 2009

Chairperson and Members Natural Area Reserves System Commission State of Hawai'i Honolulu, Hawai'i

NARS Commission Members:

SUBJECT: REQUEST FOR APPROVAL OF SPECIAL USE PERMT TO: MS. LAUREN WEISENBERGER, Ph.D. CANDIDATE, UNIVERSITY OF HWAI'I MANOA, TO CONDUCT A STUDY OF THE REPRODUCTIVE BIOLOGY OF LISTED SPECIES Schiedea nuttallii AND S. obovata IN PAHOLE NATURAL AREA RESERVE.

BACKGROUND:

These are endangered members of the Caryophyllaceae (Carnation family) and it is important to study the breeding systems of these species to determine what combination of founders should or should not be mixed at a reintroduction or augmentation; as all populations continue to decrease (not just in these species), reintroductions and augmentations are essential for preventing extinction. By determining whether or not inbreeding depression is accelerating the decline of endangered taxa and what level of mixing will increase the chance of preventing extinction, this provides the greatest chance of success.

STAFF ANALYSIS:

These listed species in Pahole are being requested because they form unique populations restricted to the Reserve and can be located with a minimum of disturbance for the studies which will include observations of floral visitors and collecting a single leaf from each plant for molecular analysis. In making observations of pollinators, arthropods may be photographed with stop-action camera and those not readily identified on site will collected to determine species.

The applicant will be issued an Endangered Plant Permit prior to conducting this work, as well as a Protected Wildlife - Invertebrate Permit for collection of insects. Staff has reviewed this application and has made recommends approval of this application, subject to a current Endangered Plant Permit, notifying staff prior to field work.

RECOMMENDATION:

That the NARS Commission approve this proposed study and to forward it to the Board of Land and Natural Resources, or its authorized representative, for their review and further action.

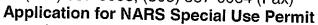
Respectfully submitted:

SETSY N. GAGNE, NARS Commission Executive Secretary

Division of Forestry and Wildlife

Department of Land and Natural Resources Division of Forestry and Wildlife

1151 Punchbowl St., Room 325; Honolulu, HI 96813 (808) 587-0063, (808) 587-0064 (Fax)





Name: Lauren Weisenberger				
Title of Proposed Activity: Pollinator Observations & Leaf Samples of Schiedea obovata &				
Schieden huitaitti				
The following activities require a Special Use Permit under HAR §13-209-5. If your work in the				
Natural Area Reserve (NAR) will involve one or more of the following, please indicate with an 'X' below:				
 X remove, injure, or kill any form of plant or animal life, except game mammals and birds hunted according to department rules* _ introduce any form of plant or animal life* _ remove, damage, or disturb any geological or paleontological features or substances* _ remove, damage or disturb any historic or prehistoric remains* _ engage in any construction or improvement* 				
engage in any construction of improvement.				
establish a temporary or permanent residence start or maintain a fire				
litter, or to deposit refuse or any other substance				
operate any motorized or nonmotorized land vehicle or air conveyance in any area (including roads and trails) not designated for its use				
 operate any motorized water vehicle of any shape or form in freshwater environments or marine waters, except as otherwise provided by DLNR's boating rules enter into, place any vessel or material on, or otherwise disturb a lake or pond 				
engage in commercial activities, defined as "the use of or activity on state lands for which compensation is received by any person for goods or services or both rendered to customers or participants in that use or activity."				
have or possess the following tools, equipments or implements: fishing gear or devices (in `Ahihi-Kina`u NAR), cutting or harvesting gear (in any NAR), and hunting gear or tools (except as permitted by the hunting rules of the department)				
like or conduct nature study with a group larger than 10				
presence in an area closed pursuant to HAR §13-209-4.5 or after visiting hours established by §13-209-4.6				
anchor any motorized or non-motorized water vehicle in the marine waters of `Ahihi-Kina`u NAR				
other (please explain):				
May require additional State or Federal permits. Applicants are responsible for identifying and securing all approvals that may be required.				
* The NARS rules and recent rule amendments can be viewed on-line at				

http://www.state.hi.us/dlnr/dofaw/Unofficial%20compilation%20HAR%2013.209.pdf

*** Please allow for a minimum permit processing time of three months***

All permits will have the following standard conditions, pursuant to HAR \S 13-209-5. Additional conditions may apply.

- 1) The permittee shall adhere to the specifications given in the permit application
- 2) Disturbance of vegetation and wildlife shall be avoided as much as possible
- 3) Precautions shall be taken to prevent introductions of plants or animals not naturally present in the area. The permittee is responsible for making sure that participants' clothing, equipment, and vehicles are free of seeds or dirt to lessen the chance of introducing any non-native plants or soil animals. Should an infestation develop attributable to the permittee, the permittee is responsible for eradication by methods specified by the department
- 4) Once approved, the permit is not transferable
- 5) Once approved, the permit does not exempt the permittee from complying with any other applicable rule or statute
- 6) The State of Hawaii shall be released and held harmless from any and all liability for injuries or death, or damage or loss of property however occurring during any activity related to the permit

I certify that the information contained in this application is true and correct.

		. 🗷	
		*	Applicant's Signature
If approved, copies of the per	mit will be provided to:		
• Applicant	viju oe provideu io.		
• NARS Commission Ex	ecutive Secretary		-
 NARS Branch staff 			
• DLNR-DOCARE	* 6		
For internal use only:			•
Application received on:		aga aga a sa agas a sa mara	·.
Distributed to District staff for			
Approval () recommended () representative on:	not recommended by NA	ARS Commission	or authorized
****	() with the ati	tached special co	nditions.
() Approved	() Not Approved		-
	pry to a description		
Chairperson, DLNR	Date	.	

Applicant Contact Information

Name: Lauren Weisenberger

If you are applying on behalf of an organization, the organization and your title:

1) This research is part of my doctoral dissertation; Investigating Inbreeding and Outbreeding Depression in Rare Species of *Schiedea* (Caryophyllaceae) on O'ahu: Implications for a Reintroduction Strategy for Recovery Efforts (University of Hawai'i, Botany Dept.)

2) This research is supported by my employer: O'ahu Army Natural Resources Program (OANRP)

Title of Proposed Activity: Pollinator Observations & Leaf Samples of Schiedea obovata & Schiedea nuttallii

Primary contact person for this permit application: Lauren Weisenberger 🏇

Mailing Address: 3903 Pili Place, Honolulu, HI 96816

Phone: (808) 226-6529 Fax: (808) 656-7471

Email: weisenbe@hawaii.edu

Supporting Information

Please provide the following information about your proposed activity that requires a special-use permit ("proposed special-use"). Failure to provide responses to the following questions may result in your application being rejected.

1. What is the period of time for which the permit is requested (e.g., the date of a proposed single event or an ongoing research project from when to when)?

* Please note: research permits are limited to one year in length, except where waived for permits to other governmental agencies where the board determines the waiver to be in the best interest of the State. Proposals for multi-year projects are advised of the need to apply for a new permit EACH year.

(Please see answer #4 for details on this study.) All visits will be scheduled in advanced with the NARS specialist, Mr. Talbert Takahama, to approve each date, in the same manner as O'ahu Army Natural Resources Program (OANRP) scheduled actions. Pollinator observations will consist of six to ten day trips in the Pahole NAR. Two sites will be visited. They are the wild population of Schiedea obovata located at the Northwest corner of West Makaleha (SchObo.LEH-B) and the new outplanting of Schiedea nuttallii below Pu'u 2210 (SchNut.PAH-E). Trips will be scheduled to the Pahole NAR between January 1st 2009 and December 31st 2009. Two people, one OANRP staff and myself will conduct visits. Two early morning and two early evening trips will be conducted (within the ten maximum). No site will be visited more than once in a given week. No one site will be visited more than five times during the year. No overnight camping will occur in the Pahole NAR. At least two of the total number of visits will be in conjunction wit scheduled monitoring by OARNP. The same people will conduct tasks of OARNP and of my research on the same day as not to add two more separate trips to the Pahole NAR. Leaf samples will be collected during two of these scheduled visits.

List the individual Natural Area Reserve(s) involved: Pahole NAR

2. Attach a map that illustrates where in the Natural Area Reserve(s) you propose to conduct your special-use. The map should be legible and reproducible in black and white. The

map should also be at the appropriate scale for the type of activity proposed and of sufficient detail to allow the Division to identify activity sites within 10 meters. For any activity off established trails, entry and exit routes should be marked.

- 3. Provide a thorough and detailed description of the proposed special use. The description should be detailed enough so that those reviewing your application understand what you propose to do and the scope of your proposal. As part of your description, please include: a) a description of the planned method of transportation to and within the Natural Area Reserve, and b) if other people than you will participate in the proposed special-use, please note how many people, and whether they are volunteers, students, research assistants, paying customers, etc...
 - a) To access the wild population of Schiedea obovata (SchObo LEH-B) at the Northwest corner of West Makaleha, two people will drive up Ka'ala Road and hike in from West Makaleha (3 Points) along the Makua fenceline. To access the Schiedea nuttallii outplanting (SchNut.PAH-E), access will be from the Pahole road. The vehicle will be an O'ahu Army Natural Resources Program (OANRP) vehicle and hiking will be along the Pahole Kahanahaiki Ridge trail. The outplanting can be accessed from this trail, there will be no hiking off this trail except around and within the outplanting site.
 - b) OANRP staff will accompany me. Neither volunteers nor students will accompany me, only OANRP staff.

For research proposals,

- c) please explain your objectives, your methods, and why the proposed special-use is necessary to your gesearch;
- d) if the research is part of your undergraduate or graduate studies, please include the name and affiliation of your major professor;
- e) if you are seeking permission to remove or introduce any form of plant or animal life, please list all species involved and specifically identify which are threatened, endangered, or candidate species.
- f) if you are seeking permission for the collection of any specimens, please note type of specimen (species and parts collected, if less than entire specimen), quantities to be collected, storage methods, and ultimate disposition.

Failure to provide sufficient information may result in your application being returned for additional information or rejected. Please feel free to attach additional sheets as necessary.

c) This work is part of my dissertation for investigating the presence or absence of inbreeding and outbreeding depression among three species of *Schiedea*; *S. obovata*, *S. kaalae*, and *S. nuttallii*. The degree of detection of inbreeding and outbreeding depression will be among all possible crosses (including self-fertilized) among each species. This information will guide suggestions as to the ideal combination of founders to utilize in a reintroduction in order to produce the most fit offspring. Offspring will be outplanted into areas within their historic range that would also represent areas suitable for establishing a reintroduction. These plantings will be monitored to measure vigor and fecundity. Vigor and fecundity will determine overall fitness. The degree of genetic variation will also be assessed using molecular approaches in conjunction with the offspring fitness and geographic distance among populations of each taxa. This is

essential for determining if the mating of more closely related individuals produce fitter or weaker progeny. If outcrossing promotes offspring fitness, the effectiveness of a mixed-stock reintroduction could potentially rely on the presence or absence of effective pollinators. Floral visitor observations could be made to help determine the dependency of each taxa on pollinator presence, the type of faunal taxa that visit each these *Schiedea*, as well as the frequency of visitations. One of the key pollinator observations that will greatly contribute to this project is the comparison of the frequency and type of pollinators present at populations with many individuals versus populations of only a few or even a single individual. This will allow helping predict the "attractiveness" of a population to potential pollinators based on their size.

In order to address these questions, I am proposing two actions within the Pahole NAR. I would like to observe floral visitors and collect leaf samples at two sites, one wild population of *Schiedea obovata* and one reintroduction site of *Schiedea nuttallii*, in the Pahole NAR. I would like to conduct pollinator observations at this wild population of *S. obovata* to use in comparison to the smaller or single-plant populations. It serves as the only wild population of *S. obovata* with more than two mature plants. These observations are critical for understanding the mating system of this taxon. Visits will consist of three to five visits at both sites to conduct pollinator observations at different times of the day and year. Three sites will be initially scheduled for both sites (six visits total), with an additional two visits to be requested for the sites where no floral visitors are observed after the first three trips. This part of my dissertation is scheduled for 2009. These visits will be in conjunction with OANRP standard monitoring of this site. Monitoring will be conducted during one of the scheduled trips for pollinator observations, as not to add one additional visit to the site. The number of staff to visit this site will not increase, as monitoring will be in conjunction with the observations.

Additionally, I would like to collect leaf samples, consisting of one leaf. Only one gram of the leaf material is necessary for the molecular analyses. The samples will be used to look for genetic variation among the other populations of these taxa. Molecular approaches to calculating genetic diversity will be utilized in conjunction with quantitative measurements such as offspring fitness. Measurements of genetic diversity will hopefully provide insight into which populations are more similar to other populations, and whether offspring produced from more genetically different parents are more fit than offspring produced from genetically similar parents. Collections made from the S.nuttallii outplanting are necessary because all stock propagated from the wild S. nuttallii in the Pahole gulch bottom is planted at this one outplanting. No stock of this particular founder is currently being grown ex situ at any nursery. Leaf samples representing the wild population of S. obovata are also essential to collect for this research. All other collections of S, obovata and most leaf collections of the other two Schiedea species will be made from first or second-generation plants propagated in a greenhouse. Much genetic diversity is lost with every generation reared in a greenhouse environment. Analyzing collections from one population of S. obovata in addition to greenhouse grown first generation plants of all possible flounders will help calculate the amount of genetic diversity lost by sampling greenhouse propagated plants. Without a wild collection, it would be impossible to determine if more genetic diversity may be present if the samples would have all been collected from wild founders.

Methodology: Observations will consist of recording the faunal taxa that visit a randomly sampled flowering plant within many ten to thirty minute blocks of time. Observations will be made several times a day, and including early morning and evening hours. Studies have

suggested that native Pyralid moths could be potential pollinators for *S. lydgatei* (Norman, Weller & Sakai 1997). This information supports the potential for evening floral visitors. No visits to any site within the Pahole NAR will begin after dark, I will always leave before it is dark. Faunal taxa, length of visit per flower and per total area surveyed, and number of flowers and plants visited per survey will be recorded at each observation. Pictures will be taken of each visitor. A small digital camera will be used to take pictures, and no camera equipment will be setup at the site. If birds are observed visiting flowers, only pictures will be taken, no birds will be collected. The method of placing a vial over the insect and letting it fly into the vial will be used to collect insects. Small insects may also be collected by aspiration. No more than two insects will be collected of any one taxon. I will be the only person to collect insects, though one other OANRP staff may take pictures as well as myself.

I will also be the only person to collect leaf samples. At the *S. obovata* population, one small leaf from one to two nodes below the apical meristem will be collected from five individuals. The five individuals will be randomly selected from a selected group of plants. These plants will be healthy and multi-branched. At the *S. nuttallii* planting, one leaf will be collected from two individuals that were vegetatively propagated from stock from the wild *S. nuttallii* population at the Pahole gulch bottom. All samples will be placed in bags of silica gel and brought to Cliff Morden's laboratory at the University of Hawai'i for DNA extraction. A molecular study to look at genetic diversity among individuals of this taxon (as well as the other two species of *Schiedea*) will be conducted using microsatellite primers indentified by Dr. Teresa Culley at the University of Cincinnati.

The reasons why this work is necessary for my research are described in my answer to question #5. All findings will be reported to the NARS Commission.

- d) This research is part of my Ph.D. dissertation. My advisor is Dr. Cliff Morden of the Botany Department at the University of Hawai'i.
- e) Specific species are uncertain at this point as floral visitors are unknown. These taxa have no known floral visitors. Due to the presence of dark, sweet nectar, however, *S. obovata* is presumed to be bird-pollinated, though no observations have been documented. Still camera pictures will be taken of birds, but no birds will be collected.
- f) Insects will be aspirated or collected into vials. A contract will be pursued with Bishop Museum to process, identify, and maintain this collection. Effort will be made to distinguish native and non-native fauna. No nets will be used to collect visitors as this could possibly damage plants. Bird visitors will be identified from pictures. The goal of these observations is to document the visitation by faunal taxa, and not to identify the exact species that effectively pollinates flowers of *S. obovata*. If individuals do produce fitter offspring through cross pollination versus selfing, planning mixed reintroductions would be pointless if there was no potential vector to move pollen from one flower to another.
- Norman, J.K., S.G. Weller & A.K. Sakai. 1997. Pollination biology and outcrossing rates in hermaphroditic *Schiedea lydgatei* (Caryophyllaceae). *American Journal of Botany* **84**(5): 641-648.

- 4. Please answer the following questions about your proposed special use:
 - a. Can your proposed special use be conducted elsewhere? If not, why not?

This project cannot be conducted elsewhere as these populations are very unique. The *S. nuttallii* outplanting is the largest site for this taxon. The wild population of this taxon does not have to be visited because the reintroduction has been successful. There are no wild or planted sites of *S. nuttallii* outside of the Pahole NAR that are as large as this outplanting. The wild population of *S. obovata* is the only population with more than two mature individuals remaining for this taxon. These larger populations are crucial to understanding if faunal taxa visit the flowers of these species of *Schiedea*. The observations will help determine how frequently potential pollinators are visiting the plants, and how likely new reintroductions will receive floral visitors. There is very little known of what visits these plant taxa, though it is assumed that they are all insect or bird pollinated (Wagner, Weller & Sakai 2005). Utilizing the larger populations of *Schiedea* in the Pahole NAR will help determine how frequently they are visited, what type of insects and birds visit flowers, whether floral visitors are native or non-native, and how much the attractiveness of a species decreases when only a few individuals remain at one site.

Additionally, the proposed leaf collections cannot be collected at other locations outside of the Pahole NAR. The stock to be collected from the S. nuttallii outplanting represents the wild founder located at the bottom of Pahole gulch. By collecting one leaf from two of the cloned individuals, this wild site does not have to be visited. This stock is only represented at this outplanting. Other founders do have ex situ representation in a greenhouse. As mentioned above, molecular analyses of genetic diversity will be compared to the quantitative fitness measurements of the offspring of the cross pollinations among populations within each species. This will help determine whether or not offspring are more fit with genetically similar or genetically distinct parents. Ideally, the most accurate source to represent to determine genetic diversity would be from the wild populations. As many populations no longer exist or have been reduced to only one or two individuals, wild populations cannot be sampled. Since most collections are therefore from first generation greenhouse stock, genetic diversity may be further reduced. By collecting samples from one wild population to compare with first generation greenhouse samples from those wild parents, I will be able to estimate the amount of diversity lost with each greenhouse grown generation, and consequently better estimate the amount of variation present among stock. The only population large enough to be sampled is the population of S. obovata at the Northwest corner of West Makaleha.

Wagner, W.L., S.G. Weller & A. Sakai. 2005. Monograph of *Schiedea* (Caryophyllaceae-Alsinoideae). *Systematic Botany Monographs* 72.

b. Is your proposed special-use consistent with the purpose and objectives of the Natural Area Reserves System (the purpose and objective of the NARS is to protect in perpetuity specific land and water areas which support communities, as relatively unmodified as possible, of the natural flora and fauna of Hawai`i)? If so, how?

The main goal of my dissertation is to determine what combination of founders should or should not be mixed at a reintroduction or augmentation of three species of *Schiedea*. As all populations continue to decrease in number, reintroductions and augmentations are essential for

preventing the extinction of these species that are currently in low numbers in the Pahole NAR. By determining whether or not inbreeding depression is accelerating the decline of endangered taxa of *Schiedea*, and what level of mixing will increase the chance of preventing extinction the greatest, we may have the chance of preventing the further loss of these species.

c. Is your proposed special-use consistent with the management plan developed for the individual Reserve(s) (Management plans are available for review at www.dofaw.net/nars or by contacting the NARS office)?

Many of the rare plants native to the Pahole NAR are protected through management efforts such as fencing, weed control, seed collection, and outplantings. Determining the representation of founders that yield the fittest, most prosperous outplantings will increase the success of these outplantings. Successful outplantings will help in the Pahole NAR's goals to perpetuate the rare species of *Schiedea* found in this precious habitat.

d. Does your proposed special-use provide a benefit (direct or indirect) to the Natural Area Reserves System or to the individual Reserve(s) or both? (For research, please note whether any studies have previously been made similar to the one proposed and how you will convey your research findings to the Department).

The results of this research have an immediate effect of the management of these three species of *Schiedea*, all of which occur in the Pahole NAR. Conclusions drawn from this study will directly affect how this taxon is reintroduced into areas both within and adjacent to the Pahole NAR. Reintroduction guidelines will be developed from this research and the O'ahu Army Natural Resources Program will apply these guidelines.

e. Will the proposed special-use damage or threaten to damage the integrity or condition of the natural, geological, or cultural resources in the individual Natural Area Reserve(s) and adjacent area or region? If so, how? If not, why not?

No. The special-use permit is to observe floral visitors and collect leaf samples and small insects. There will not be an impact to the land at either location or the surrounding area. Additionally, the population of *S. obovata* is located at the perimeter of the Pahole NAR and access will be from adjacent state Forest Reserve land and not through the Pahole NAR.

f. Does the proposed special-use comply with the provisions and guidelines contained in HRS Chapter 205A, entitled 'Coastal Zone Management,' where applicable? HRS Chapter 205A can be accessed at:

http://www.capitol.hawaii.gov/hrscurrent/Vol04_Ch0201-0257/HRS0205A/
Yes – this research is not located at a coastal zone and there is no impact to any coastal environment.

g. Have you (the applicant) previously received a NARS Special Use Permit? If so, did you comply with the conditions of any previously approved permit (including providing a final report as requested)?

No, this is the first NARS Special Use Permit for which I am applying.

h. Do you (the applicant) have any other current NARS special-use permits? If so, please list and state whether you are currently in compliance with the conditions of those permits.

Yes, I am currently in compliance with the following NARS special-use permits:

- 1. O'ahu Army Natural Resources Program: Ka'ena, Ka'ala, & Pahole NAR
- 5. Is the proposed special-use expected to have an environmental impact on the Natural Area Reserve(s) or the surrounding area? If, so please elaborate. If not, why not? Please include discussion of any off-trail work, such as mist-netting, setting of traps, removal of vegetation, etc. and any measures planned to mitigate any short and long-term damage.

Several insects may be removed from the two habitats where the floral visitor observations will be conducted. Some faunal visitors will attempt to be identified from still-camera photography to the family level. Smaller visitors will be collected or aspirated into vials. Bishop Museum staff will be contracted to process, identify and maintain collected insects. It cannot be assumed that this insect removal will not have any environmental impact. It is doubtful, however, that the removal of several insects will affect the rate of pollination for these two sites. The leaf samples to be collected at the West Makaleha S. obovata population will only be one leaf from five healthy, branched individuals. Samples would not be collected if there were any reason, past research, or opinion that this leaf removal could hinder the viability of the plant. No studies or personal communication with horticulturists have suggested that this leaf removal could be damaging to the sampled plants.

- 6. There is an application fee of \$50 to cover the cost of processing; please attach a check made out to: *Department of Land and Natural Resources*.
- 7. For research proposals, please list any local collaborators or contacts (if any).
- 1. Dr. Cliff Morden, University of Hawai'i, Department of Botany: 808-956-9636
- 2. Kapua Kawelo, O'ahu Army Natural Resources Program: 808-655-9178